ORIGINAL ARTICLE

Asian Herpetological Research 2021, 12(2): 167–177
DOI: 10.16373/j.cnki.ahr.200084

A New Species of *Trimeresurus* Lacépède, 1804 (Squamata: Viperidae) from Southwestern China, Vietnam, Thailand and Myanmar

Zening CHEN[1,2#], Shengchao SHI[1,2#], Jun GAO[3,4], Gernot VOGEL[5], Zhaobin SONG[1], Li DING[2*] and Rong DAI[3,4*]

[1] *Key Laboratory of Bio-Resource and Eco-Environment of Ministry of Education, College of Life Sciences, Sichuan University, Chengdu 610065, Sichuan, China*
[2] *Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, Sichuan, China*
[3] *Nanjing Institute of Environmental Sciences, Ministry of Ecology and Environment of the People's Republic of China, Nanjing 210042, Jiangsu, China*
[4] *State Environmental Protection Key Laboratory of Biosafety, Ministry of Ecology and Environment of China, Nanjing 210042, Jiangsu, China*
[5] *Society for Southeast Asian Herpetology, Im Sand 3, D-69115 Heidelberg, Germany*

Abstract The pit vipers of the genus *Trimeresurus* Lacépède, 1804 is one of the largest groups of Asian snakes, distributed from India to China and Indonesia. Recent surveys in Jiangcheng and Simao, Yunnan Province, China resulted in a new species previously allocated to *T. albolabris*. Combining morphological and molecular data, we describe it as *Trimeresurus guoi* **sp. nov.** The new species morphologically differs from *T. albolabris* in the yellow green ventral color; an indistinct ventrolateral line; the absence of a postocular stripe; the firebrick-red iris; a dark red stripe on dorsal tail; hemipenes with relatively weak sparse papillae, reaching 23rd subcaudal when unextruded. Molecularly, the new species forms a clearly divergent lineage (BPP 1.00/ UFB 100). Uncorrected pairwise distances of mitochondrial gene Cyt *b* between the new species and other known species of the subgenus *Trimeresurus* range from 0.052 (*T. albolabris*) to 0.071 (*T. insularis*).

Keywords morphology, phylogenetics, taxonomy, *Trimeresurus*

# Both authors contributed equally to this paper.
*Corresponding author: Prof. Li DING, from Chengdu Institute of Biology, Chinese Academy of Sciences, China, with his research focusing on ophiology, molecular ecology and biogeography; Dr. Rong DAI, from Nanjing Institute of Environmental Sciences, Ministry of Ecology and Environment of the People's Republic of China, with her research focusing on biodiversity conservation and nature reserve management.
E-mail: dingli@cib.ac.cn (DING L.); dairong@nies.org (DAI R.)
Received: 28 July 2020 Accepted: 5 September 2020

1. Introduction

The Asian green pit vipers (*Trimeresurus* Lacépède, 1804) are a complex and species-rich group of venomous snakes (Gumprecht *et al.*, 2004; Malhotra and Thorpe 2004; Vogel *et al.*, 2004; Guo *et al.*, 2015). There are 50 known species, but the diversity of the genus *Trimeresurus* is still underestimated (Wostl *et al.*, 2016; Mulcahy *et al.*, 2017; Chen *et al.* 2019; Chen *et al.* 2020; Uetz *et al.*, 2020; Mirza *et al.*, 2020). *Trimeresurus albolabris* (Gray, 1842) is widely recorded in Southern China, and adjacent Southeastern Asia (Zhu, *et al.*, 2016). Two subspecies of this species have been raised to full species *T. insularis* and *T. septentrionalis* based on molecular evidence (Giannasi *et al.*, 2001). Furthermore, Chen *et al.* (2020) described *T. caudornatus* based on populations previously identified as *T. albolabris*. Mirza *et al.* (2020) described *T. salazar*, which is similar to *T. septentrionalis* and *T. albolabris*. Besides, Zhu *et al.* (2016) indicate that *T. albolabris* from Southern China and Southeastern Asia forms a clade containing five lineages with significant divergence. These indicate that *T. albolabris* is a species complex.

During several field surveys in Yunnan Province in 2019, a series of specimens resembling *T. albolabris* were collected from Jiangcheng and Simao, Yunnan Provinces, China. However, subsequent morphological and molecular analysis revealed these specimens represent a species different from *T. albolabris* and all other recognized congeners. Herein, it is described as a new species.

2. Materials and Methods

2.1. Sampling Ten individuals of the new species were collected in the field, including six road kills and two individuals kept alive. Specimens were fixed and stored in 80% ethanol and deposited at the Chengdu Institute of Biology (CIB), Chengdu, China. Living individuals are kept in the laboratory of CIB, and their saliva was collected for molecular analysis.

2.2. Morphologic analysis The description is based on morphological characters regarded as taxonomically significant by Vogel *et al.* (2004). Body and tail length were measured with a tape ruler to the nearest 1 mm. Other measurements were conducted with a digital caliper to the nearest 0.1 mm. Body length (SVL) and tail length (TaL) were taken with a string, which was then measured using a scale. Ventral scales were counted according to Dowling (1951). The number of dorsal scale rows are given at one head length behind head, at midbody, and at one head length before vent, respectively. Cephalic scales (Cep) were counted on a straight line between the middle of the supraoculars. Abbreviations used in the description: HL: head length (from the tip of the snout to corner of mouth); SVL: snout-vent length; TaL: tail length; TL: total length. ED: the horizontal eye diameter. DSR: dorsal scale row; IL: infralabial scale; SC: subcaudal scale; SL: supralabial scale; VEN: ventral scale. Color description was made according to wiki color-coding. Sex is determined by examination of the hemipenes.

The abbreviations for institutions and comparative material examined are presented in Appendix I.

2.3. Molecular analysis Total genomic DNA was isolated from liver specimens or saliva stored in 95% ethanol using the EasyPure® Genomic DNA kit (TransGen Biotech, Beijing China). Genomic DNA was visualized using 1.0% agarose gel electrophoresis and stored in –20°C. We amplified fragments of mitochondrial cytochrome b (Cyt *b*), NADH dehydrogenase subunit 4 (ND4), 12S ribosomal RNA (12S) and 16S ribosomal RNA (16S) genes using the primer pairs L14910/H16064 (Burbrink *et al.*, 2000), ND4/Leu (Parkinson *et al.*, 2000), L1091/H1557 (Knight and Mindell, 1993), 16Sar–L/16Sbr–H (Parkinson, *et al.*, 2000), respectively. PCR amplifications were performed in 25 µL reactions: 1.0 µL genomic DNA, 1 µL light strand primer (10 µmol/L), 1.0 µL heavy strand primer (10 µmol/L), 12.0 µL I-5™-2 × High-Fidelity Master Mix (Beijing Qingke New Industry Biotechnology Co., Ltd), 10.0 µL ddH$_2$O. Polymerase Chain Reaction (PCR) under the following conditions: initial denaturation for 2 min at 95 °C, followed by 35 cycles: denaturation at 94 °C for 40 s, annealing temperature 48 °C for Cyt *b*, 56 °C for ND4, 52 °C for 12S and 54 °C for 16S for 25 s, elongation at 72 °C for 15 s, and then finalized with elongation step of 2 min at 72 °C, with a PTC-100 thermal cycler (BioRad, USA). All PCR products were visualized using 1.0% agarose gel electrophoresis. Successful targeted PCR products were then outsourced to Beijing Qingke New Industry Biotechnology Co., Ltd. for PCR purification, cycle sequencing, and sequencing. Details of sequences and GenBank accession numbers are presented in supporting files (Table S1).

Taxa for molecular phylogenetics were selected based on the tree topologies recovered by Zhu *et al.* (2016) and Chen *et al.* (2020). GenBank accession numbers are presented in supporting files (Table S1). The sequences were aligned by Mega 7 (Kumar *et al.*, 2016), checked by eyes, and adjusted if necessary. The optimal models of sequence evolution for both genes and codons were identified by Partition finder 2.1.1 under Bayesian Information Criterion (BIC) (Lanfear *et al.*, 2012). Phylogenetic trees were constructed based on a concatenated dataset using maximum likelihood (ML) and Bayesian inference (BI). The ML analysis was conducted by using IQ-TREE (Nguyen *et al.*, 2015) with following models: GTR+ I+Γ was the best-fit for the 12S,16S, and the first codon position of Cyt *b* and ND4, HKY+I for the second codon position of Cyt *b* and ND4, and GTR+G for the third codon position of Cyt *b* and ND4. Ultrafast Bootstrap Approximation (UFB; Hoang *et al.*, 2017) using 5000 bootstrap replicates to assess node support. Nodes with UFB values of 95 and above were considered significantly supported (Hulsenbeck *et al.*, 2001; Wilcox *et al.*, 2002). The BI analysis was conducted by using MrBayes 3.2 (Ronquist *et al.*, 2012) with the same models of ML analysis. Two simultaneous runs were performed with four chains, three hot and one cold. The simulation ran for 10 million generations, was sampled every 1000 generations using the Markov Chain Monte Carlo (MCMC). The first 1000 trees were discarded as burn-in, and posterior probabilities were determined from the remaining trees. Nodes with Bayesian posterior probabilities (BPP) of 0.95 and above were considered well supported (Hulsenbeck *et al.*, 2001; Wilcox *et al.*, 2002). *Azemiops feae* and *Protobothrops elegans* were used as outgroup. After removing outgroup taxa, MEGA7 (Kumar *et al.*, 2016) was used to calculate uncorrected pairwise sequence divergence between the species.

3. Results

Taxonomy

***Trimeresurus guoi* sp. nov. Chen, Shi, Vogel, and Ding**

Figures 1–3

ZooBank No.: 103364A6-75A4-435E-B1A4-7A899F3FEA93

Holotype. YNJC0012, adult male, a roadkill, collected from Jiangcheng County, Yunnan Province (22.5738°N, 101.6095° E) on 9 September 2019 (Figure 1).

Paratype. One adult female DL2019111703 (alive), two juvenile

females JCR2019062401, DL2019090630; three adult males DL2019111701, DL2019090530 and DL2019090830; and three juvenile males YNJC0010, JCR2019061901, DL2019111702. Detailed information is presented in Table 1.

Diagnosis. (1) Dorsal body jungle-green with faint transverse dark bands on skin, ventral body yellow green. (2) Lateral head jungle-green above lower margin of eyes, and green yellow below, without postocular stripes. (3) Ventrolateral line of male yellow-green, narrow, only present on outermost row of dorsal scales. (4) Iris firebrick-red in both sexes. (5) First supralabial fused with nasal. (6) Head scales feebly keeled; dorsal scale row 23-21-15 (N = 10), feebly keeled except the outermost rows; ventral scale 154–163 in males (N = 6), 158–160 in females (N = 3); subcaudal scale 58–72 in males (N = 7), 52–59 in females (N = 3). (7) Hemipenes long, reaching $23^{rd}/32^{nd}$ subcaudals when unextruded/extruded, papillae relatively weak and sparse.

Description of holotype (Figure 1). Body elongated; head triangular, slightly damaged, distinct from neck; head length 34.6 mm, 4.8% of snout-vent length; eyes large, the horizontal eye diameter 13.4% of head length, pupil vertical.

Snout-vent length : 513 mm; Tail length : 142 mm; Total length : 655 mm; Tail length/Total length : 21.7 %.

Ventral scale: 158; Subcaudal scale: 69, all paired; anal shield entire.

Dorsal scale row: 23-21-15 scales, rhomboid, feebly keeled, gradually smoother towards ventrals, first row smooth.

Rostral trapezoidal in front view, base twice as broad as top and 1.6 times of height, front edge protruding and nearly rounded, visible in profile. Nasals large, sub-rectangular, undivided, partially fused with the first upper labial. Internasals trapezoidal, width twice of height, edged with four small scales, and separated from supraoculars by four small scales, in contact with each other. Three canthal scales bordering the canthus rostralis present between the internasal and corresponding supraoculars, not larger than adjacent snout scales. Scales on snout dorsal small, smooth, irregular in shape. Twelve Cephalic scales presents between supraoculars. Occipital scales rhomboid, flat, feebly keeled. Temporals rhomboid, slightly larger, mostly feebly keeled, two most outer rows smooth.

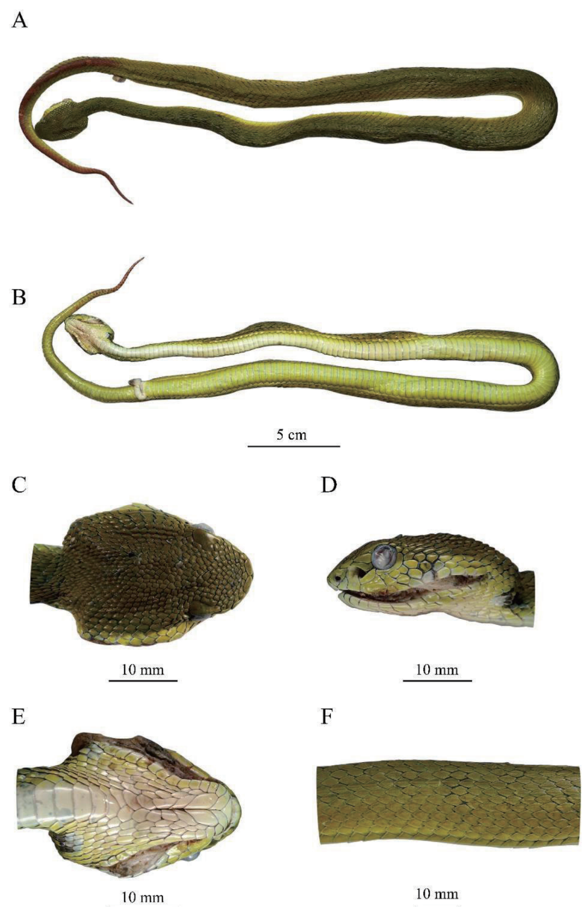

Figure 1 Holotype (adult male YNJC0012) of *Trimeresurus guoi* sp. nov. in preservation. Dorsal (A) and ventral (B) view of the specimen; dorsal (C), lateral (D) and ventral (E) view of head; lateral view of middle body (F). Photographed by Shengchao SHI after two months preservation in 80% ethanol.

Table 1 Main morphological characters of *Trimeresurus guoi* **sp. nov**. Abbreviations are listed in the Materials and Methods. "–" indicated the data missing.

Taxa	Voucher	Sex	DSR	VEN	SC	SL left / right	IL left / right	Cep	SVL	TaL	TL	TaL/TL
T. guoi **sp. nov.**	YNJC0012	M	23-21-15	158	69	10/–	12/–	11	513	142	655	0.22
T. guoi **sp. nov.**	DL2019111703	F	23-21-15	160	59	11/11	13/13	10	743	147	890	0.17
T. guoi **sp. nov.**	JCR2019062401	F	23-21-15	155	69	11/10	11/11	10	334	81	415	0.20
T. guoi **sp. nov.**	YNJC0010	M	23-21-15	158	52	10/10	11/11	10	354	68	422	0.16
T. guoi **sp. nov.**	DL2019090630	F	– -21-15	160	55	–	–	–	358	76	434	0.18
T. guoi **sp. nov.**	JCR2019061901	M	– -21-15	156	66	–	–	–	292	76	368	0.21
T. guoi **sp. nov.**	DL2019090530	M	– -21-15	155	70	–	–	–	495	133	628	0.21
T. guoi **sp. nov.**	DL2019090830	M	23-21-15	163	72	10/10	12/12	–	444	123	567	0.22
T. guoi **sp. nov.**	DL2019111701	M	23-21-15	154	68	10/9	11/12–	10	454	125	579	0.22
T. guoi **sp. nov.**	DL2019111702	M	23-21-15	162	58	11/10	12/12	10	363	60	423	0.14

One loreal present on both sides. Two elongated preoculars above the loreal pit, the lower form upper margin of loreal pit. Two postoculars present on both lateral sides of head. Supraoculars entire, with eight/ten small scales bordering left/right supraoculars. One subocular present on both lateral sides of head, elongated and crescent-shaped, surrounding nearly half of eyes from loreal to lower postoculars, with eleven scales bordered on left.

Ten supralabials present on left. First supralabial triangular, small. Second supralabial separated from prelacunal. Third supralabial largest, wider than high. Fourth supralabial wider than high, separated from subocular by a scale as large as temporals. Fifth supralabial separated from subocular by two scales the same size. Sixth SL similar in size to the first, smaller than second to fourth. Two tiny scales present above the third SL, below subocular. Twelve infralabials on left. The first pair of infralabials contact each other in ventral view. Anterior three infralabials in contact with chin shields.

Coloration in preservation (Figure 1). Description based on holotype when preserved in 80% ethanol for three months. Dorsal head and body olive, body with faint black transverse bands with a width of approximately one to three dorsal scales.

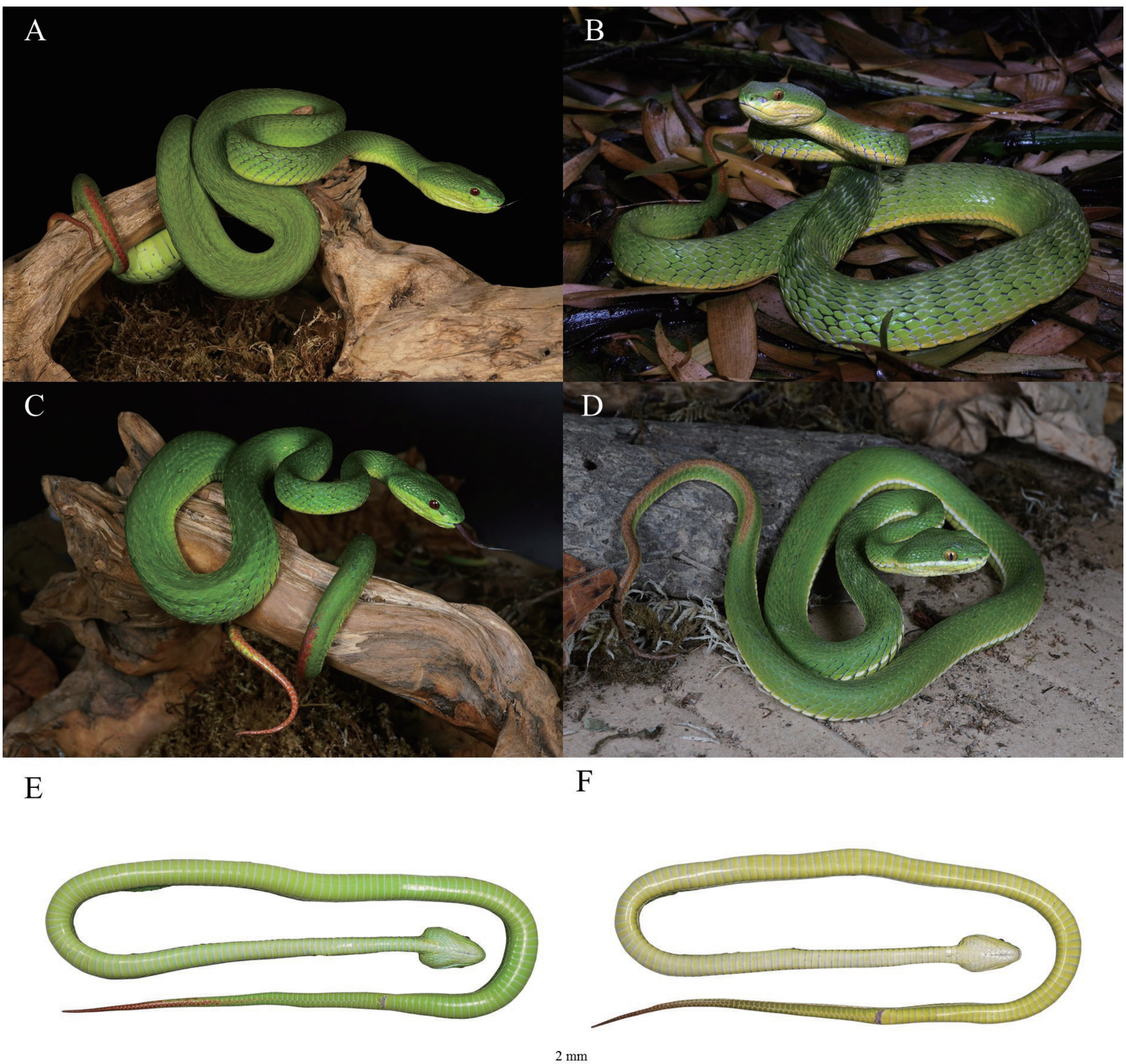

Figure 2 Comparison of coloration between *Trimeresurus guoi* **sp. nov** (Left) and *T. albolabris* (Right). A and B: Different body pattern (yellow green vs. multicolor) in female; C and D: Different ventrolateral line (absent vs. present) and postocular stripe (absent vs. present) in male; E and F: Different ventral (yellow green vs. yellow). Specimens of *T. albolabris* compared were colleted from Guanghzou, Guangdong Province, China. Photographed by Shengchao SHI except B by Liang ZHANG.

Lateral head olive above lower margin of eye, greenish yellow below. The color of lateral body gradually turns from olive near spine to olive-yellow on first row of dorsal scales. Ventral head yellowish white. Ventrals after head light yellowish white, gradually turning yellowish green towards vent. Dorsal tails mostly dark red, inner two rows of dorsal tail scales dark red, other dorsal scales olive. Most subcaudal scale yellowish green, posterior 30% gradually dark red.

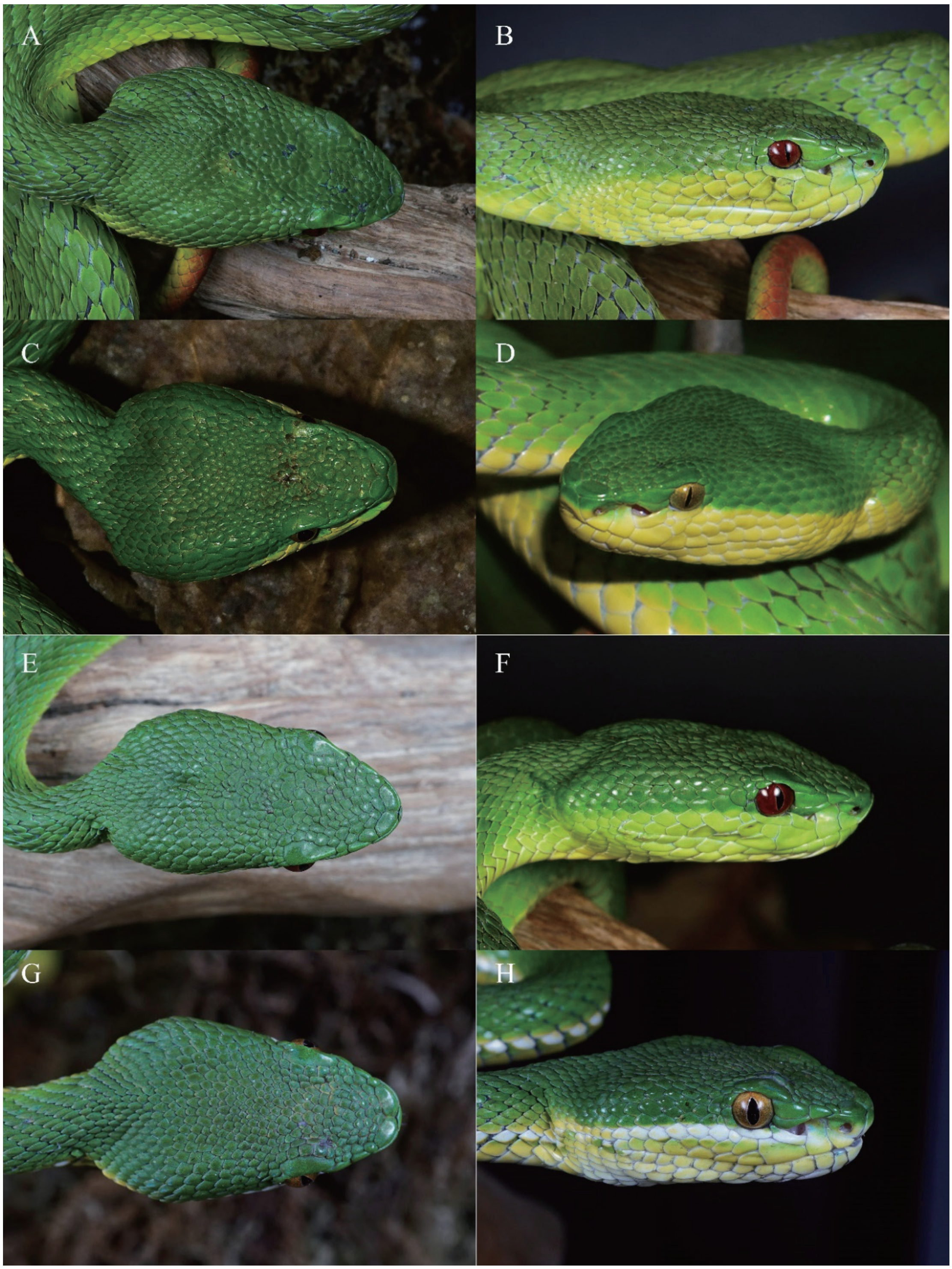

Figure 3 Comparison in head scales and coloration between *Trimeresurus guoi* **sp. nov.** and *T. albolabris*. Left, doral view of head; right, lateral view of head. A, B, female of *T. guoi* **sp. nov.**; C, D, female of *T. albolaris*; E, F, male of *T. guoi* **sp. nov.**; G, H, male of *T. albolaris*. A, C, E and G: Different head shape (more elongated skull in new species) in both genders; B and D: Different iris color (firebrick-red vs. copper) in female; F and H: Different iris color (firebrick-red vs. copper) and postocular stripe (absent vs. present) in male. Specimens of *T. albolabris* compared were colleted from Guangzhou, Guangdong Province, China. Photographed by Shengchao SHI except D by Liang ZHANG.

Coloration in life (Figures 2–3). Description based on three males DL2019111701, DL 2019111702 DL2019090830. Dorsal head and body jungle green. Lateral head jungle-green above lower margin of eyes, and green-yellow below, without postocular stripes. Dark transverse bands the width of one to three dorsal scales present on body,. Lateral body mostly jungle-green, gradually lighter from spine to ventrals. First row of dorsal scales grass-green, with a narrow green-yellow longitudinal stripe, which forms a faint green-yellow ventrolateral line. Ventral body yellow-green, becoming lighter in color anteriorly. Tail with a dark red longitudinal stripe in inner two rows of dorsal scales, other dorsal scales on tail jungle-green. Most SC green-yellow, posterior one third gradually dark red. Iris firebrick-red, pupils edged with lighter color. Tone coloration maroon.

Hemipenis (Figure 4 left). Description based on male paratype DL2019111701 (SVL 454 mm). Hemipenis elongated, reaching 23rd/32nd subcaudal when unextruded/extruded; bifurcates at 6th subcaudal, covered with weak sparse papillae after the bifurcation to about 11th subcaudal. Skin on hemipenis after 11th subcaudal feebly calyculate. The *sulcus spermaticus* shallow, divides near the base of the organ.

Intraspecific morphological variation. All ten specimens were of similar body coloration and body shape. Females without ventrolateral line (Figure 2A), outermost dorsal scales yellow green. Males with relatively longer tail and more subcaudal scales than females. Supralabials vary between 10 or 11 (N = 10); Infralabial vary from 11 to 13 (N = 10); Cephalic scales vary from 9 to 11 (N = 8) (More details in Table 1).

Comparison. The new species is morphologically and phylogenetically (see below) referred to the subgenus *Trimeresurus* (Malhotra and Thorpe, 2004; David *et al*., 2009), and is morphologically similar to *T. albolabris*, *T. septentrionalis*, and *T. caudornatus*. See main characters separating it from these three species in Table 2.

Trimeresurus guoi **sp. nov.** is distinguishable from *T. albolabris* as follows (Figures 2–4): (1) Ventral body yellow green vs. creamy white or light yellow. (2) Ventrolateral line unicolor, indistinct, yellow-green, present only on first row of most dorsal scales of males vs. multicolor, distinct in males, basically white, upper margin light yellow, lower margin fern-green, present on first two rows of dorsal scales. (3) Postocular stripe absent

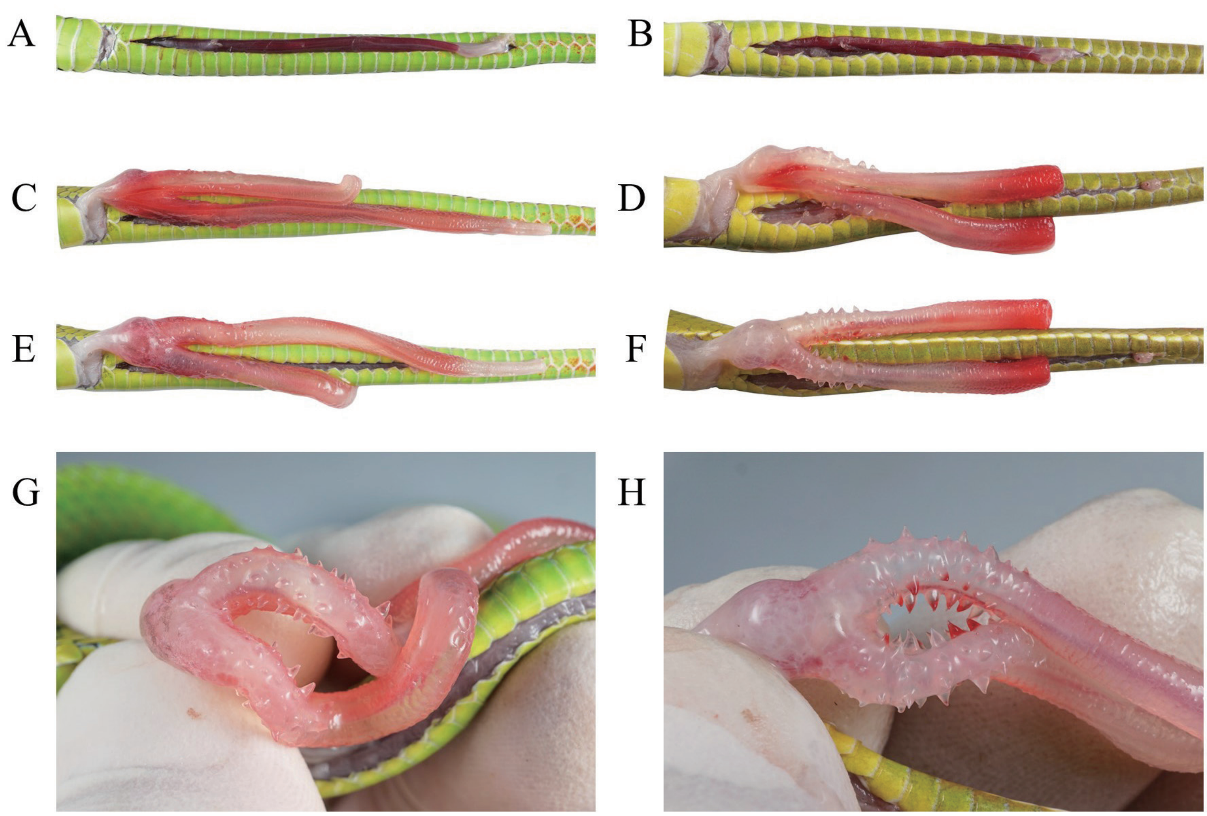

Figure 4 Comparison in hemipenis between *Trimeresurus guoi* **sp. nov.** (Left) and *T. albolabris* (Right). A and B: fresh unextruded hemipenis (longer in new species); C and D sulcate view of fresh extruded hemipenis (longer in new species); E and F: asulcate view of fresh extruded hemipenis (longer in new species); G and H: spines after bifurcation (papillae relatively small and sparse vs. papillae pointy, well developed and dense). Photographed by Shengchao SHI.

Table 2 Summary of selected characters for members of similar species.

Species	Dorsal coloration	Color of lateral stripe on head	Color stripe on ventral tail	Dorsal scale rows at midbody	TaL/TL	Ventrals	Subcaudals
T. albolabris	Green	White	Absent	21	0.14/0.21	150–160	51–66
T. cf. *albolabris*	Green	White	Absent	21	0.14/0.22	151–164	49–67
T. septentrionalis	Green	White	Absent	21	0.19/0.24	164–181	55–83
T. caudornatus	Green	Absent	Present	21	0.15/0.19	161–163	52–72
T. guoi **sp. nov.**	Green	Absent	Absent	21	0.14/0.22	154–163	52–72

vs. white postocular stripe extending from loreal pit to lateral body. (4) Iris firebrick-red vs. iris copper. (5) Stripe on dorsal tail dark red vs. stripe on dorsal tail bronze. (6) Hemipenis longer, reaching 23rd subcaudals when unextruded, papillae relatively small and sparse vs. hemipenis relatively shorter, reaching 18th subcaudals when unextruded, papillae pointy, well developed and dense (Figure 4H).

Trimeresurus goui **sp. nov.** is distinct from *T. septentrionalis* by: (1) Fewer ventrals, 154–163 in males (N = 7), 158–160 in females (N = 3) vs 164–170 in males (N = 5) and 166–171 in females (N = 13). (2) Fewer SC, 58–72 in males (N = 7), 52–59 in females (N = 3) vs. 74–80 in males (N = 5) and 56–66 in females (N = 13). (3) Lateral head jungle-green above lower margin of eyes, and green-yellow below, without postocular stripes, iris firebrick-red vs. head below the eye green, only slightly or not lighter, iris yellow.

Trimeresurus guoi **sp. nov.** is distinct from *T. caudornatus* by: (1) DSR 23–21–15 vs DSR 21/22–21–15. (2) Iris firebrick-red in both genders vs. iris golden yellow in both genders. (3) The orange-red stripe along the ventral part of the tail absent vs. present. (4) Hemipenis relatively shorter, reaching 32nd subcaudal when extruded vs. reaching 37th or 38th subcaudal when extruded.

Trimeresurus guoi **sp. nov.** is distinct from *T. salazar* by: (1) Postocular stripe absent in both genders vs. reddish orange postocular stripe extending from loreal pit to lateral body in male. (2) Ventrolateral line yellow-green in male and absent in female vs. yellow with a faint orange in males and yellow in females. (3) Iris firebrick-red in both genders vs. iris golden yellow in both genders.

Trimeresurus guoi **sp. nov.** is distinct from *T. insularis* by: (1) Dorsal scale row feebly keeled except the outermost rows vs. strongly keeled expect the first row. (2) Lateral head jungle-green above lower margin of eyes, and green-yellow below vs. head green above, blue-green, or light green (derived from photos in Vogel 2006). (3) Geographically isolated from type locality of the latter (continental, from southwestern China and adjacent area vs. islands of Soe and Timor, Indonesia).

Trimeresurus guoi **sp. nov.** is distinct from *T. erythrurus* by: (1) Scales in 21 rows at mid-body vs. usually 23–25 longitudinal rows. (2) Head scales feebly keeled vs. strongly keeled. (3) Ventrolateral stripe absent in females vs. a thin white line present in most females.

Trimeresurus guoi **sp. nov.** is distinct from *T. purpureomaculatus* by the following characters: (1) Scales in 21 longitudinal rows at mid-body vs. 25–27 (N = 29). (2) Head scales feebly keeled vs. strongly keeled. (3) Body jungle-green vs. usually not green (except some specimens from Sumatra). (4) Lateral head jungle-green above lower margin of eyes, and green yellow below vs. upper labials usually not lighter than body but in some populations yellow. (5) Fewer Cephalic scales, 10–11(N = 6) vs. 13–18 (N = 11).

Trimeresurus guoi **sp. nov.** is distinct from *T. macrops* by: (1) No postocular stripe vs. prominent lateral white or light blue stripe in males. (2) Ventrolateral stripe indistinct, faint green yellow vs. prominent white lateral stripes in males. (3) Eyes relatively smaller the horizontal eye diameter/head length 13.4 % (vs. the horizontal eye diameter/head length 16.1%). (4) Head oval, gradually widening behind eyes (vs. head triangular, widens abruptly behind eyes).

Trimeresurus guoi **sp. nov.** differs from *T. rubeus and T. cardamomensis* principally by the same characters as from *T. macrops*. It furthermore differs from *T. cardamomensis* by the iris color being firebrick-red rather than golden yellow.

Trimeresurus guoi **sp. nov.** is different from *T. cantori* by: (1) Fewer dorsal scale row (21 vs. 25–29). (2) Fewer supralabial scale (10–11 vs. 11–13, only very exceptionally 11. (3) Fewer Cephalic scales (10–11 vs. 13–17). (4) Fewer ventrals (155–163 vs. 170–182).

Trimeresurus guoi **sp. nov.** is distinct from *T. fasciatus* by: (1) Dorsal body jungle-green with faint transverse dark bands on skin vs. brownish grey with olivaceous brown or dark brown crossbands on the back (David *et al*., 2003). (2) Ventral body yellow-green dorsal tail dark red vs. greyish brown or brown. (3) Internasals broad trapezoidal, broadly in contact vs. separated from each other by one scale. (4) *Trimeresurus fasciatus* is endemic to Tanahjampea Island.

Trimeresurus guoi **sp. nov.** is distinct from *T. andersoni* by: (1) Dorsal body jungle-green with faint transverse dark bands on skin vs. color above and below variable, usually brown, buff, or blackish. (2) Fewer dorsal scale row at midbody (21 vs. 23 to 25, rarely 21). (3) Fewer ventrals (154–163 vs. 171–183) (Gumprecht *et al*., 2004). Besides, *T. andersoni* is endemic to the Andaman and

Nicobar Islands.

Trimeresurus guoi **sp. nov.** is distinct from *T. labialis*, *T. honsonensis* Grismer, Ngo and Grismer, 2008, *T. venustus*, and *T. kanburiensis* by body color and pattern. The dorsal body of *T. guoi* **sp. nov.** jungle-green with faint transverse dark bands on skin vs. brown in *T. labialis* (Vogel *et al.*, 2014), green patterned with spotted brownish speckles in *T. honsonensis*, *T. venustus* (Vogel *et al.*, 1991), and *T. kanburiensis* (David *et al.*, 2004).

Etymology. The specific name is in honor of Dr. Peng Guo (Sichuan, China), the first researcher on the taxonomy and systematics of the genus *Trimeresurus sensu lato* through molecular analysis in China. We suggest the following common names as "Guo's green pit viper" in English and "Diān Nán Zhú Yè Qīng (滇南竹叶青)" in Chinese.

Phylogenetic analysis

Our DNA dataset contained 56 specimens with 2582 base pairs. The Bayesian Inference (BI) and Maximum likelihood analysis (ML) recovered trees bearing consistent phylogenetic structure (Figure 5). Phylogenetically, the specimens from Yunnan province form a clearly divergent lineage with samples from Thailand (AM A165 and AM A229), Vietnam (ROM 39389) and Myanmar (CAS 222595) (Figure 5, BPP 1.00/UFB 100), with mitochondrial Cyt *b* divergence 0.046–0.128 from all previously known species (Table S2). This divergence is clearly among inter-species level since the uncorrected p-distance are 0.031 between *T. erythrurus* and *T. cantori* and 0.041 between *T. cantori* and *T. andersoni*. Taking into consideration the morphology of those specimens from Thailand, Vietnam and Myanmar, we hypothesize that these individuals belong to the new species based on the short branch length and low pairwise distance between those from Jiangcheng County and Simao District, Yunnan Province, China (< 0.019, Table S2).

Due to the lectotype (BMNH 1946.1.19.85) of *T. albolabris* being collected from Hong Kong, China (Wallach *et al.*, 2014), and the sequences from Hong Kong (AM A157), southern China and

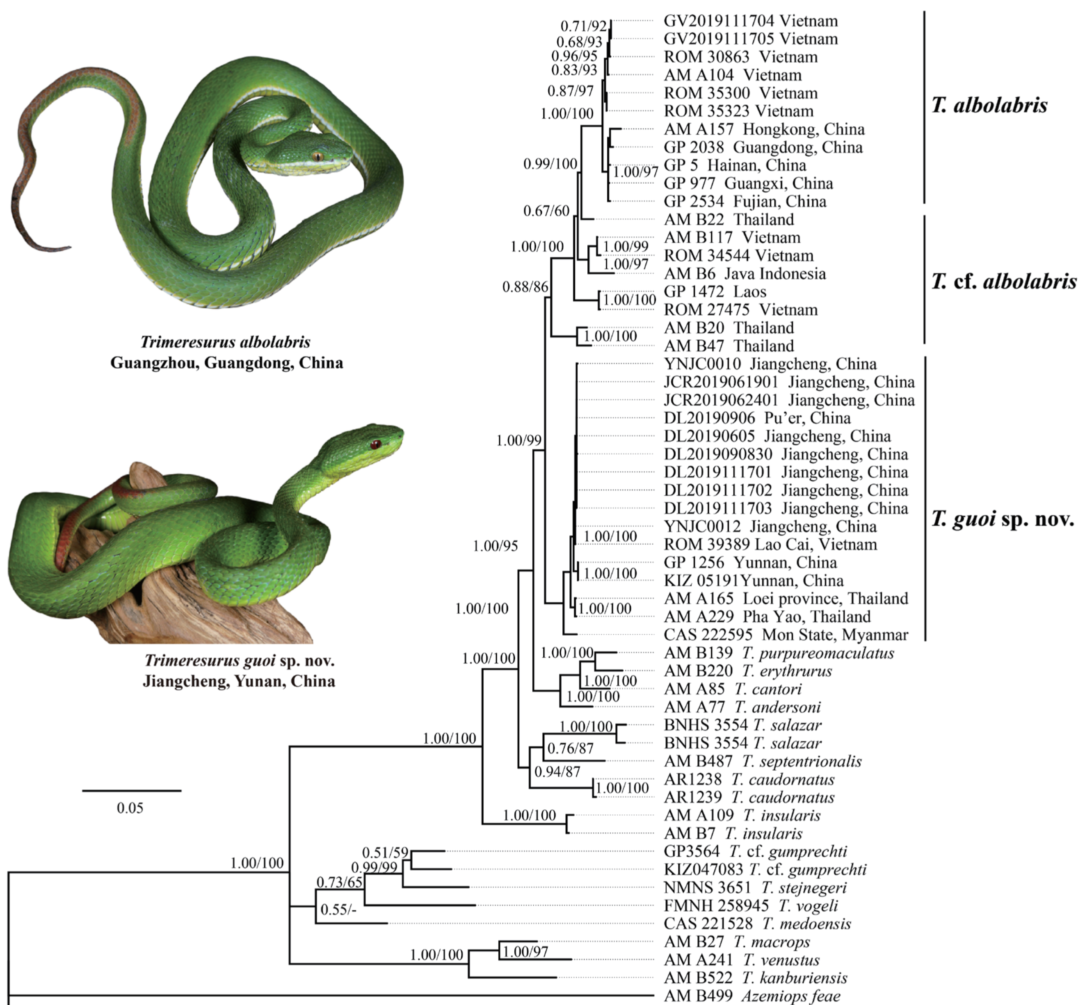

Figure 5 Bayesian Inference (BI) and maximum likelihood (ML) analyses trees of the genus *Trimeresurus* based on Cyt *b*, ND4, 12S, and 16S. The major bootstrap and posterior probability values (BPP/UFB) were presented (the ones lower than 50% denoted as "-").

northern Vietnam forms a monophyletic group with strong support (Figure 5, UFB 100/ BPP 1.00), we treat those specimens as *T. albolabris*. However, these specimens from Thailand (AM B22), Vietnam (ROM 27475), Laos (GP 1472), and Java (AM B6) were outlying to the *T. albolabris*, the deep divergences, both intra-species (0.000–0.049, Table S2) and inter-species (>0.029, Table S2), indicated that specimens are cryptic. We therefore refer to these specimens as *T.* cf. *albolabris* and their taxonomic status remains unresolved.

The uncorrected sequence divergence in the studied Cyt *b* fragment showed the new species to be divergent from the previously recognized species by interspecific genetic distances of 0.048–0.061 for *T. albolabris*, 0.034–0.049 for *T.* cf. *albolabris*, 0.045–0.125 all other known species (Table S2).

Distribution and habitat. *Trimeresurus guoi* **sp. nov.** is known from Jiangcheng County and Simao District, Southern Yunnan Province, China, and adjacent Vietnam (Lao Cai), Thailand (Loei and Pha Yao) and Myanmar (Mon State) (Figure 6). It is found in tropical monsoon forests with clearly defined dry and rainy seasons, at elevations reaching approximately 1,400 m in Jiangcheng, Yunnan, China (Figure 7).

Figure 6 Distribution of *Trimeresurus guoi* **sp. nov.**, *T. albolabris* and *T.* cf. *albolabris*.

4. Discussion

The Asian green pit vipers are notoriously difficult to classify due to highly similar squamation morphology. Specimens of the new species were treated as *T. albolabris* for a long time. However, multiple evidence including molecular and morphological data based on fresh and living specimens collected near or from the type locality showed that *T. guoi* **sp. nov.** differs from *T. albolabris* as described above. Furthermore, several lineages in the subgenus *Trimeresurus* need to be reexamined in detail and might lead to the description of other new species.

Figure 7 Habitat of *T. guoi* **sp. nov.** in Jiangcheng, Yunnan Province, China.

The description of *T. guoi* **sp. nov.** once more emphasizes the underestimation of the diversity of green pitvipers in Yunnan, where six species were recorded: *T. yunnanensis*, *T. gumprechti*, *T. stejnegeri*, *T popeiorum*, *T. yingjiangensis*, and *T. caudornatus* (Guo *et al.*, 2015; Chen *et al.*, 2019; Chen *et al.*, 2020, this paper). Yunnan Province is located in the southeastern-most corner of the Tibetan Plateau, with high mountains and deep valleys, which may cause isolation and accelerate diversification. According to Figure 6, the Red River might be a boundary of the new species and *T. albolabris*.

Ecological differences can drive speciation (McKelvy and Burbrink, 2017). *T. guoi* **sp. nov.** and *T. albolabris* occupy different ecological niches. Specimens of *T. albolabris* collected from Hong Kong and Guangdong were found in southern subtropical monsoon forests at low elevation (altitude under 500 m) with annual mean temperatures of 21.5–22.5 °C (Xu *et al.*, 2007). In contrast to this, *T. guoi* **sp. nov.** was found in tropical monsoon forests with clearly defined dry and rainy seasons and a higher elevation (reaching above 1,400 m), the annual mean temperature is 14.8–18.9 °C (Liu, 2000). Further studies on how ecological differences drive speciation of these two species are recommended.

Acknowledgements This project was funded by the Biodiversity Investigation, Observation and Assessment Program of Ministry of Ecology and Environment of China (2019–2023) and the Natural Science Foundation of Jiangsu Province, China (BK20160103). We thank Patrick

Campbell (BMNH), Jens Vindum and Alan Leviton (CAS), Andreas Schmitz (MHNG), Alain Dubois and Annemarie Ohler (MNHN), Silke Schweiger, Richard Gemel and Georg Gassner (NMW), Pim Arntzen and Esther Dondorp (RMNH, Leiden), Mark-Oliver Rödel and Frank Tillack (ZMB), Jakob Hallermann (ZMH, Hamburg) for data about the collections under their care. We thank Liang Zhang and Hangzhang Cai for providing photographs. We especially thank Tom Charlton for the language editing.

References

Blyth E. 1846. Notes on the fauna of the Nicobar Islands. J Asiatic Soc Bengal, 15: 367–379

Burbrink F. T, Lawson R., Slowinski J. B. 2000. Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. Evolution, 54: 2107–2118

Chen Z. N, Zhang L., Shi J. S., Tang Y. Z., Guo Y. H., Song Z. B., Ding L. 2019. A new species of the genus *Trimeresurus* from Southwest China (Squamata: Viperidae), Asian Herpetol Res, 10(1): 13–23

Chen Z. N., Yu J., Vogel G., Shi S. C., Song Z. B., Tang Y. Z., Yang J., Song Z. B., Ding L., Chen C. S. 2020. A new pit viper of the genus *Trimeresurus* (Lacépède, 1804) from Southwest China (Squamata: Viperidae). Zootaxa, 4768(1): 112–128

David P., Ineich I. 1999. Les serpents venimeux du monde: systématique et répartition. Dumerilia, Paris, 3: 3–499

David P., Petri M., Vogel G., Doria G. 2009. A new species of pitviper of the genus *Trimeresurus* (*Popeia*) from northern Sumatra (Reptilia, Squamata, Viperidae). Annali del Museo Civico di Storia Naturale “G. Doria”, 100: 323–346

David P., Vogel G., Vidal N. 2003. On *Trimeresurus fasciatus* (Boulenger, 1896) (Serpentes: Crotalidae), with a discussion on its relationships based on morphological and molecular data. Raffles B Zool, 51: 149–157

David P., Vogel G., Sumontha M., Pauwels O. S. G., Chanhome L. 2004. Expanded description of the poorly known pitviper *Trimeresurus kanburiensis* Smith, 1943, with confirmation of the validity of *Trimeresurus venustus* Vogel, 1991 (Reptilia: Serpentes: Crotalidae). Russ J Herpetol, 11:81–90

Dowling H. G. 1951. A proposed standard system of counting ventrals in snakes. Brit J Herpetol, 1:97–99

Figueroa A., Mckelvy A. D., Grismer L. L., Bell C. D., Lailvaux S. P. 2016. A species-level phylogeny of extant snakes with description of a new colubrid subfamily and genus. Plos One, 11, 1–31

Giannasi N., Thorpe R. S., Malhotra A. 2001. The use of amplified fragment length polymorphism in determining species trees at fine taxonomic levels: analysis of a medically important snake, *Trimeresurus albolabris*. Mol Ecol, 10: 419–426

Gray J. E. 1842. Synopsis of the species of rattle-snakes, or family of Crotalidae. Zool Misc, 2: 47–51

Grismer L. L., Grismer J. L., McGuire J. A. 2006. A new species of pit viper of the genus *Popeia* (Squamata: Viperidae) from Pulau Tioman, Pahang, West Malaysia. Zootaxa, 1305, 1–19

Gumprecht A., Tillack F., Orlov N., Captain A., Ryabov S. 2004. Asian Pit vipers. Geitje Books, Berlin, 368

Guo P., Zhang F. 2001. Comparative studies on hemipenes of four species of *Trimeresurus* (*sensu stricto*) (Serpentes: Crotalinae). Amphibia-Reptilia, 22: 113–117

Guo P., Liu Q., Zhong G., Zhu F., Yan F., Tang T., Xiao R. 2015. Cryptic diversity of green pit vipers in Yunnan, south-west China (Squamata, Viperidae). Amphibia-Reptilia, 36: 265–276

Hoang D. T., Chernomor O., von Haeseler A., Minh B. Q., Le S. V. 2017. UFBoot2: Improving the ultrafast bootstrap approximation. Mol Biol Evol, 35: 518–522

Huelsenbeck J. P., Ronquist F., Nielsen R., Bollback J. P. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science, 294: 2310–2314

Knight A., Mindell D. P. 1993. Substitution Bias, Weighting of DNA Sequence Evolution, and the Phylogenetic Position of Fea’s Viper. Syst Biol, 42:18–31

Knowles L. L., Carstens B. C. 2007. Delimiting species without monophyletic gene trees. Syst Biol, 56: 887–895

Kramer E. 1977. Zur Schlangenfauna Nepals. Rev Suisse Zool, 84: 721–761

Kuhl H. 1824. Sur les reptiles de Java. Extrait d’une lettre adressée de Java en Hollande, par M. Kuhl, datée de Pjihorjavor au pied du Pangerango, le 18 juillet 1821 (Algem. Konst en Letterbode; 1822). Bulletin des Sciences naturelles et de Géologie (Section II du Bulletin Universel des Sciences naturelles), Paris, 2: 79–83

Kumar S., Steche G., Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol, 33: 1870

Lacépède B. G. E. 1804. Mémoire sur plusieurs animaux de la Nouvelle Hollande dont la description n’a pas encore été publiée. Annales du Muséum d’Histoire naturelle, Paris, 4:184–211, pls 55–58

Lanfear R., Calcott B., Ho S. Y. W., Guindon S. 2012. Partition Finder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol Biol Evol, 29: 1695–1701

Liu W. J. 2000. Impact of climatic variation on net primary productivity of natural vegetation in Xishuangbanna in recent 40 years. J Mt Sci-Engl, 4: 296–300 [In Chinese]

Malhotra A., Thorpe R. S. 2004. A phylogeny of four mitochondrial gene regions suggests a revised taxonomy for Asian pit vipers (*Trimeresurus* and *Ovophis*). Mol Phylogenet Evol, 32: 83–100

McKelvy A. D., Burbrink F. T. 2017. Ecological divergence in the yellow-bellied kingsnake (*Lampropeltis calligaster*) at two North American biodiversity hotspots. Mol Phylogenet Evol, 106: 61–72

Mirza Z. A., Bhosale H. S., Phansalkar P. U., Sawant M., Gowande G. G., Patel H. 2020. A new species of green pit vipers of the genus *Trimeresurus* Lacépède, 1804 (Reptilia, Serpentes, Viperidae) from western Arunachal Pradesh, India. Zoosyst Evol, 96(1): 123–138 [①]

Nguyen L. T., Schmidt H. A., von Haeseler A., Minh B. Q. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating

① “Arunachal Pradesh” Refers to as southern Tibet - an indigenous territory of the People's Republic of China in terms of history, geography and international law.

maximum likelihood phylogenies. Mol Biol Evol, 32: 268–274

Parkinson C. L., Zamudio K. R., Greene H. W. 2000. Phylogeography of the pit viper clade *Agkistrodon*: historical ecology, Species status, and conservation of cantils. Mol Evol, 9: 411–420

Pope C. H., Pope S. H. 1933. A study of the green pit-vipers of southeastern Asia and Malaysia, commonly identified as *Trimeresurus gramineus* (Shaw), with description of a new species from Peninsular India. Am Mus Novit, 620: 1–12

Pyron R. A., Kandambi H. K., Hendry C. R., Pushpamal V., Burbrink F. T., Somaweera R. 2013. Genus-level phylogeny of snakes reveals the origins of species richness in Sri Lanka. Mol Phylogenet Evol, 66: 969–978

Regenass U., Kramer E. 1981. Zur Systematik der grünen Grubenottern der Gattung *Trimeresurus* (Serpentes, Crotalidae). Rev Suisse Zool, 88: 163–205

Ronquist F., Teslenko M., Mark P. V. D., Ayres D., Darling A., Hohna S., Larget B., Liu L., Suchard M. A., Huelsenbeck J. P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol, 61: 539–542

Sanders K. L., Malhotra A., Thorpe R. S. 2006. Combining molecular, morphological and ecological data to infer species boundaries in a cryptic tropical pit viper. Biol J Linn Soc, 87: 343–364

Schlick-Steiner B. C., Steiner F. M., Seifert B., Stauffer C., Christian E., Crozier R. H. 2010. Integrative Taxonomy: a multisource approach to exploring biodiversity. Annu Rev Entomol, 55: 421–438

Sharma S. K., Pandey D. P., Shah K. B., Tillach F., Chappuis F., Thapa C. L., Alirol E., Kuch U. 2013. Venomous Snakes of Nepal: A Photographic Guide. B.P. Koirala Institute of Health Sciences, Dharan, Nepal, 71 pp

Smith M. A. 1943. The Fauna of British India, Ceylon and Burma, including the whole of the Indo-Chinese subregion. Reptilia and Amphibia. Vol. III, Serpentes. Taylor and Francis, London, xii + 583 pp

Stamatakis A., Hoover P., Rougemont J. 2008. A rapid bootstrap algorithm for the RaxML web-servers. Syst Biol, 75: 758–771

Sumontha M., Kunya K., Pauwels O. S. G., Nitikul A., Punnadee S. 2011. *Trimeresurus* (*Popeia*) *phuketensis*, a new pit viper (Squamata: Viperidae) from Phuket island, southwestern Thailand. Russ J Herpetol, 18: 185–194

Taylor E. H. 1965. The serpents of Thailand and adjacent waters. University of Kansas Science Bulletin 45: 609–1096

Theobald W. 1868. Catalogue of Reptiles in the Museum of the Asiatic Society of Bengal, 1866. J As Soc Bengal, 88: i-iii + 88 pp, pls 1–4

Uetz P., Freed P., Hošek J. 2019. The Reptile Database, Retrieved from http://www.reptile-database.org

Vijayakumar S. P., David P. 2006. Taxonomy, Natural History, and Distribution of the Snakes of the Nicobar Islands (INDIA), based on new materials and with an Emphasis on endemic species. Russ J Herpetol, 13:11–40

Vogel G. 1991. Eine neue *Trimeresurus*-Art aus Thailand, *Trimeresurus venustus* sp. nov. (Reptilia: Serpentes: Crotalidae). Sauria, 13: 23–28

Vogel G., David P., Chandramouli S. R. 2014. On the systematics of *Trimeresurus labialis* Fitzinger in Steindachner, 1867, a pitviper from the Nicobar Islands (India), with revalidation of *Trimeresurus mutabilis* Stoliczka, 1870 (Squamata, Viperidae, Crotalinae). Zootaxa, 3786: 557–573

Vogel G., David P., Pauwels O. S. G. 2004. A review of morphological variation in *Trimeresurus popeiorum* (Serpentes: Viperidae: Crotalinae), with the description of two new species. Zootaxa, 727: 1–61

Warrell D. A., Looareesuwan S., Stimson A. F., Hutton R. A. 1992. Rediscovery and redefinition of Malcolm Smith's *Trimeresurus kanburiensis* in Thailand, with a report of envenoming. T Roy Soc Trop Med H, 86: 95

Wilcox T. P., Zwickl D. J., Heath T. A., Hillis D. M. 2002. Phylogenetic relationships of the Dwarf Boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Mol Phylogenet Evol, 25: 361–371

Wostl E., Sidik I., Trilaksono W., Shaney K. J., Kurniawan N., Smith E. N. 2016. Taxonomic Status of the Sumatran Pitviper *Trimeresurus* (*Popeia*) *toba* David, Petri, Vogel and Doria, 2009 (Squamata: Viperidae) and Other Sunda Shelf Species of the Subgenus Popeia. J Herpetol, 50: 633–641

Xu Z. Y., Sun W., Luo S. B., Li Y., Jiang H. R. 2007. The variation characteristics for temperature of 3 cities of Guangdong province in the past one hundred years. J South China Norm Univ (Nat Sci Edi), 2: 137–142 [In Chinese]

Yang D. T., Rao D. Q. 2008. Amphibia and Reptilia of Yunnan. Yunnan Science and Technology Press, Kunming, 365–366 pp [In Chinese]

Zhao E. M. 2006. Snakes of China. Anhui Science and Technology Publishing House, Hefei, 139–140 pp [In Chinese]

Zhao E. M., Huang M. H., Zong Y. 1998. Fauna Sinica, Reptilia, Squamata. Serpentes, Vol. 3. Science Press, Beijing, 445–451 pp [In Chinese]

Zhao E. M., Yang D. T. 1997. Amphibians and Reptiles of the Hengduan Mountains Region. Science Press, Beijing, 286–286 pp [In Chinese]

Zhu F., Liu Q., Che J., Zhang L., Chen X., Yan F. 2016. Molecular phylogeography of white-lipped tree viper (*Trimeresurus*; Viperidae). Zool Scr, 45: 252–262

Handling Editor: Chen YANG

How to cite this article:

Chen Z. N., Shi S. C., Gao J., Vogel G., Song Z. B., Ding L., Dai R. A New species of *Trimeresurus* Lacépède, 1804 (Squamata: Viperidae) from Southwestern China, Vietnam, Thailand and Myanmar. Asian Herpetol Res, 2021, 12(2): 167–177. DOI: 10.16373/j.cnki.ahr.200084

Appendix

Institution acronyms

BMNH, Natural History Museum (Formerly the British Museum (Natural History)), London, UK. CAS, California Academy of Sciences, San Francisco, USA. MNHN, Muséum national d'Histoire naturelle, Paris, France. NHMB, Naturhistorisches Museum Basel, Swizerland. MHNG, Muséum d'histoire naturelle, Ville de Genève, Switzerland. NMW, Naturhistorisches Museum Wien, Austria. RMNH: Rijksmuseum van Natuurkijke Historie, Leiden, Netherlands. SMF, Natur-Museum und Forschungs-Institut Senckenberg, Frankfurt-am-Main, Germany. ZMB, Zoologisches Museum für Naturkunde der Humboldt-Universität zu Berlin, Berlin, Germany. ZMH, Zoologisches Museum Hamburg [Formerly Zoologisches Institut und Museum], Universität Hamburg, Hamburg, Hamburg, Germany. ZSI, Zoological Survey of India, Kolkata [Calcutta], West Bengal, India.

Comparative material examined:

Trimeresurus albolabris. (19 specimens) China. BMNH 1946.1.19.85, BMNH 1946.1.23.73 (Syntypes) "China". MNHG 1464.88–89 "Tung Kum, Canton". NMW 23927, "Koksingas Port". NMW 23905:2, 23905:5–7, "Hainan, Ting-An". NMW 23626.4–5 "Hongkong". DL000245, DL000248, "Canton". ZMB 27669 "S-Kuang-tung". ZMB 52600, "Fung Wan". ZMB 66282 "Lu Kung, Katon". ZMB 66283 "N-Kuantung". Vietnam. CIB GV2019111704–5, "Tam Dao".

Trimeresurus cf. albolabris. (29 specimens) Vietnam. NMW 23901.8 "Phuc-Son, Annam". NMW 23904.3–5, MNW 23920.7 "Annam". MNW 23920.3 "Saigon". Thailand. NMW 19528 "Thailand". NMW 23901:3–4 "Dom Rek". NMW 23926.1, NMW 23926.6–9, NMW 23930.1–2 "Pu-Kin". NMW 27946.2–3, 27946.5–6 "Hills of Bangkok". NMW 23898.1–2 "Don-Pia-Fei". ZMB 70196 "Surat Thani". Indonesia. NMW 23901.6, 23926.1–3"Java". MNW 23902 "Tasikmalaja, W Java". RMNH 17189 "Sumatra".

Trimeresurus septentrionalis (18 specimens) Nepal. CAS 135750 (Paratype) "Nähe Pokhara". MHNG 1404.31 (Holotype) MHNG 1400.18, 24–26, 29–32, 34–39, 45, 47 (all Paratypes) "Nähe Pokhara".

Trimeresrus insularis (7 specimens). Indonesia NHMB 12773 (Holotype) "Soe, Timor". NMHW 39581 "Bali". MNHN 4056, "Timor Island". MNHN 4057, "Indes Orientales". MNHN 2002.0402, "Wetar Island". SMF 76352, 76353, "Flores Island".

Trimeresrus erythrurus. (22 specimens) India BMNH 1940.3.9.22 "Naga Hills". NMHW 23903:1–2, Guwahati, Assam. ZSI 3052, ZSI 3002, ZSI 3013, ZSI 3045–46" Samagooting, Assam", ZMH R-6933 "Himalaya" Myanmar BMNH 61.10.2.5–6, 1908.6.23.96 "Rangoon". ZMH R-6934" Rangoon". CAS 220377, 240036, 204989 "Rakhin". CAS 239352, 239502, 239511, 40120 "Ayeyarwaddi State". CAS 240120 "Kakhim State ". CAS 243175 "Magway".

Trimeresurus fasciatus (4 specimens): BMNH 96.4.29.46 (Holotype), "Jampea Island", now Tanahjampea, Province of Sulawesi Selatan, Indonesia. - MNHN 1999.9071, MNHN 2002.0401-02, Tanahjampea, Province of Sulawesi Selatan, Indonesia, through the pet trade.

Table S1 GenBank numbers for sequences used in this study.

No.	Taxa	Voucher	Locality	Cyt *b*	ND4	12S	16S	References
1	*T. albolabris*	AM A104	Vin Phuc, Vietnam	AY352769	AY352837	AY352803	AY352742	Malhotra and Thorpe, 2004
2	*T. albolabris*	AM A157	Hong Kong, China	AF171884	AY352839	AY352805	AY352744	Malhotra and Thorpe, 2004
3	*T. albolabris*	GP 5	Hainan, China	KP999410	KP999504			Zhu *et al.*, 2016
4	*T. albolabris*	GP 977	Guangxi, China	KP999420	KP999514			Zhu *et al.*, 2016
5	*T. albolabris*	GP 2038	Guangdong, China	KP999388	KP999482			Zhu *et al.*, 2016
6	*T. albolabris*	GP 2534	Fujian, China	KP999403	KP999497			Zhu *et al.*, 2016
7	*T. albolabris*	ROM 30863	Vinh Phu, Vietnam	KP999429	KP999523			Zhu *et al.*, 2016
8	*T. albolabris*	ROM 35300	Hai Duong, Vietnam	KP999435	KP999528			Zhu *et al.*, 2016
9	*T. albolabris*	ROM 35323	Cao Bang, Vietnam	KP999438	KP999531			Zhu *et al.*, 2016
10	*T. albolabris*	GV2019111704	Tam Dao, Vietnam	MN746390	MN746402	MN746414	MN746426	This study
11	*T. albolabris*	GV2019111705	Tam Dao, Vietnam	MN746391	MN746403	MN746415	MN746427	This study
12	*T.* cf. *albolabris*	AM B117	Ho Chi Minh city, Vietnam	AF517190	AF517222	AF517166	AF517179	Malhotra and Thorpe, 2004
13	*T.* cf. *albolabris*	AM B20	Nakhon Si Thammarat, Thailand	GQ428474	GQ428481	GQ428491	GQ428465	Malhotra *et al.*, 2010
14	*T.* cf. *albolabris*	AM B22	Nonthaburi, Thailand	AF517189	AF517221	AF517165	AF517178	Malhotra and Thorpe, 2004
15	*T.* cf. *albolabris*	AM B47	Phetburi province, Thailand	AF517187	AF517216	AF517160	AF517173	Malhotra and Thorpe, 2004
16	*T.* cf. *albolabris*	AM B6	Cilacap, Java, Indonesia	AF517186	AF517213	AF517158	AF517171	Malhotra and Thorpe, 2004
17	*T.* cf. *albolabris*	ROM 34544	GiaLai, Vietnam	AY352770	AY352838	AY352804	AY352743	Malhotra and Thorpe, 2004
18	*T.* cf. *albolabris*	ROM 27475	Kontum, Vietnam	KP999428	KP999522			Zhu *et al.*, 2016
19	*T.* cf. *albolabris*	GP 1472	Sekong, Laos	KP999370	KP999464			Zhu *et al.*, 2016
20	*T. guoi* **sp. nov.**	YNJC0010	Jiangcheng Country, Yunnan, China	MN746392	MN746404	MN746416	MN746428	This study
21	*T. guoi* **sp. nov.**	YNJC0012	Jiangcheng Country, Yunnan, China	MN746393	MN746405	MN746417	MN746429	This study
22	*T. guoi* **sp. nov.**	JCR2019061901	Jiangcheng Country, Yunnan, China	MN746394	MN746406	MN746418	MN746430	This study
23	*T. guoi* **sp. nov.**	JCR2019062401	Jiangcheng Country, Yunnan, China	MN746395	MN746407	MN746419	MN746431	This study
24	*T. guoi* **sp. nov.**	DL20190906	Simao, Yunnan, China	MN746396	MN746408	MN746420	MN746432	This study
25	*T. guoi* **sp. nov.**	DL20190605	Jiangcheng Country, Yunnan, China	MN746397	MN746409	MN746421	MN746433	This study
26	*T. guoi* **sp. nov.**	DL2019090830	Jiangcheng Country, Yunnan, China	MN746398	MN746410	MN746422	MN746434	This study
27	*T. guoi* **sp. nov.**	DL2019111701	Jiangcheng Country, Yunnan, China	MN746399	MN746411	MN746423	MN746435	This study
28	*T. guoi* **sp. nov.**	DL2019111702	Jiangcheng Country, Yunnan, China	MN746400	MN746412	MN746424	MN746436	This study
29	*T. guoi* **sp. nov.**	DL2019111703	Jiangcheng Country, Yunnan, China	MN746401	MN746413	MN746425	MN746437	This study
30	*T. guoi* **sp. nov.**	AM A165	Loei province, Thailand	AF517185	AF517214	AF517169	AF517182	Malhotra and Thorpe, 2004
31	*T. guoi* **sp. nov.**	AM A229	Pha Yao province, Thailand	AY059566	AY059583	AY059544	AY059560	Malhotra and Thorpe, 2004
32	*T. guoi* **sp. nov.**	CAS 222595	Mon State, Myanmar	KP999354	KP999449			Zhu *et al.*, 2016
33	*T. guoi* **sp. nov.**	GP 1256	Yunnan, China	KP999368	KP999462			Zhu *et al.*, 2016
34	*T. guoi* **sp. nov.**	KIZ 05191	Yunnan, China	KP999424	KP999518			Zhu *et al.*, 2016
35	*T. guoi* **sp. nov.**	ROM 39389	Lao Cai, Vietnam	KP999440	KP999533			Zhu *et al.*, 2016
36	*T. purpureomaculatus*	AM B139	Perak state, Malaysia	AY352771	AY352840	AY352806	AY352745	Malhotra and Thorpe, 2004
37	*T. erythrurus*	AM B220	Chittagong district, Bangladesh	AY352768	AY352834	AY352800	AY352739	Malhotra and Thorpe, 2004
38	*T. cantori*	AM A85	Nicobar Islands, India	AF171889	AY352836	AY352802	AY352741	Malhotra and Thorpe, 2004
39	*T. andersoni*	AM A77	Andaman Islands, India	AF171922	AY352835	AY352801	AY352740	Malhotra and Thorpe, 2004
40	*T. septentrionalis*	AM B487	Kathmandu district, Nepal	AY352755	AY352818	AY352784	AY352724	Malhotra and Thorpe, 2004
41	*T. caudornatus*	AR1238	Yingjiang Country, Yunnan,China	MK575036	MK575038	MK575040	MK575042	Chen *et al.*, 2020
42	*T. caudornatus*	AR1239	Yingjiang Country, Yunnan,China	MK575037	MK575039	MK575041	MK575043	Chen *et al.*, 2020
43	*T. salazar*	BNHS 3554	Tibet, China				MN684366	Mirza *et al.*, 2020
44	*T. salazar*	BNHS 3555	Tibet, China		MN686204		MN684365	Mirza *et al.*, 2020
45	*T. kanburiensis*	AM B522	Kanchanaburi, Thailand	AY289225	AY289231	AY289219	AY352737	Malhotra and Thorpe, 2004
46	*T. insularis*	AM A109	Java, Indonesia	AY352767	AY352833	AY352799	AY352738	Malhotra and Thorpe, 2004
47	*T. insularis*	AM B7	Timor, Indonesia	AY059568	AY059586	AY059534	AY059550	Malhotra and Thorpe, 2004
48	*T. macrops*	AM B27	Bangkok, Thailand	AF517184	AF517219	AF517163	AF517176	Malhotra and Thorpe, 2004
49	*T. venustus*	AM A241	Nakhon si Thammarat, Thailand	AF171914	AY293930	AY293931	AY352723	Malhotra and Thorpe, 2004
50	*T. medoensis*	CAS 221528	Kachin, Myanmar	AY352765	AY352831	AY352797	AY352735	Malhotra and Thorpe, 2004
51	*T. stejnegeri*	NMNS 3651	Fujian, China	AF277677	AY059594	AY059541	AY059557	Malhotra and Thorpe, 2004
52	*T.* cf. *gumprechti*	GP3564	Mengzi, Yunnan, China	KT216389	KT216438	KT216298	KT216344	Malhotra and Thorpe, 2004
53	*T.* cf. *gumprechti*	KIZ047083	Jingdong, Yunnan, China	KT216398	KT216447	KT216306	KT216351	Malhotra and Thorpe, 2004
54	*T. vogeli*	FMNH 258945	Champassak, Laos	AY059581	AF517225	AF517170	AF517183	Malhotra and Thorpe, 2004
55	*Protobothrops elegans*	UMMZ199970	Ishigaki Is., Ryukyu Is., Japan	AY223575	U41893	AF057201	AF057248	Malhotra and Thorpe, 2004
56	*Azemiops feae*	AM B499	China	AY352747	AY352808	AY352774	AY352713	Malhotra and Thorpe, 2004

Table S2 Uncorrected pairwise distances between *Trimeresurus* species based on 1044 base pairs from the mitochondrial genes Cytb. Specimens *T. guoi* **sp. nov.** are in bold font.

No.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*T. albolabris*	0.006 (0.000–0.018)															
2	*T.* cf. *albolabris*	0.042 (0.023–0.061)	0.029 (0.000–0.049)														
3	*T. guoi* **sp. nov**	**0.052 (0.048–0.061)**	**0.042 (0.034–0.049)**	0.005 (0.000–0.019)													
4	*T. purpureomaculatus*	0.062 (0.061–0.068)	0.057 (0.051–0.061)	**0.045 (0.041–0.051)**													
5	*T. erythrurus*	0.086 (0.071–0.092)	0.078 (0.075–0.081)	**0.060 (0.050–0.069)**	0.012*												
6	*T. cantori*	0.074 (0.067–0.077)	0.056 (0.061–0.067)	**0.046 (0.042–0.049)**	0.006*	0.031											
7	*T. andersoni*	0.065 (0.058–0.068)	0.059 (0.057–0.067)	**0.053 (0.050–0.056)**	0.041	0.061	0.041										
8	*T. septentrionalis*	0.083 (0.081–0.085)	0.071 (0.064–0.081)	**0.068 (0.063–0.071)**	0.072	0.086	0.073	0.074									
9	*T. caudornatus*	0.082 (0.086–0.096)	0.075 (0.071–0.078)	**0.069 (0.065–0.079)**	0.068	0.078	0.066	0.066	0.069								
10	*T. insularis*	0.098 (0.091–0.104)	0.083 (0.078–0.087)	**0.071 (0.065–0.073)**	0.069	0.086	0.079	0.081	0.089	0.085							
11	*T. gumprechti*	0.142 (0.140–0.145)	0.139 (0.137–0.141)	**0.130 (0.128–0.136)**	0.128	0.128	0.152	0.142	0.127	0.139	0.142						
12	*T. stejnegeri*	0.138 (0.128–0.142)	0.134 (0.130–0.139)	**0.126 (0.121–0.129)**	0.119	0.147	0.130	0.127	0.126	0.137	0.110	0.056					
13	*T. vogeli*	0.140 (0.139–0.148)	0.137 (0.129–0.142)	**0.147 (0.138–0.149)**	0.139	0.150	0.139	0.128	0.134	0.136	0.135	0.087	0.093				
14	*T. medoensis*	0.118 (0.115–0.121)	0.110 (0.103–0.114)	**0.115 (0.109–0.120)**	0.116	0.132	0.120	0.120	0.113	0.122	0.111	0.079	0.076	0.079			
15	*T. macrops*	0.133 (0.127–0.136)	0.127 (0.122–0.132)	**0.125 (0.118–0.131)**	0.112	0.139	0.122	0.122	0.127	0.128	0.132	0.113	0.104	0.120	0.095		
16	*T. venustus*	0.133 (0.121–0.137)	0.134 (0.125–0.139)	**0.146 (0.137–0.149)**	0.134	0.163	0.141	0.137	0.148	0.145	0.133	0.107	0.113	0.127	0.098	0.043	
17	*T. kanburiensis*	0.135 (0.132–0.138)	0.135 (0.128–0.142)	**0.125 (0.123–0.128)**	0.123	0.134	0.120	0.125	0.132	0.135	0.130	0.108	0.107	0.128	0.118	0.055	0.066

Note: * Because of the poor sequence quality of *T. purpureomaculatus*, the genetic distance between *T. purpureomaculatus* and *T. erythrurus* and *T. cantori* are low.